Exhibit A

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High throughput identification of specific small molecule ligands of therapeutic RNA targets from a diverse collection of small molecules

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Although the rich structural and functional diversity of RNA, often compared to that of proteins, makes RNA a likely target for small molecules (1), the lack of an efficient method for identification of novel small molecule ligands of a given RNA target has been a limitation to this approach. We have developed a high throughput method to Screen for Compound with Affinity for Nucleic acid targets, SCAN, which allows the rapid identification of specific small molecule ligands of any target RNA from a random compounds library. Here we describe SCAN and its application to the discovery of antiviral compounds that bind to the encapsidation signal of the hepatitis B virus.

INTRODUCTION

Regulation of gene expression and other metabolic pathways by mechanisms that involve structured RNA motifs is ubiquitous among eukaryotic cells as well as infectious agents such as bacteria and viruses. It is well established that small molecules that bind RNA can inhibit RNA function. For example, 2-DOS-containing aminoglycosides bind specifically to the structured HIV Rev response element, RRE, blocking its recognition by the Rev protein, and thereby inhibiting HIV replication (2). However, the lack of efficient methods for the identification of novel small molecule ligands of RNA has hampered efforts to exploit the vast diversity of therapeutic RNA targets. Thus, the development of effective technologies for the discovery of novel ligands of therapeutic RNA targets is highly desirable. In this report we describe the development of SCAN, a high throughput assay for the identification of small molecule ligands of any structured RNA target from random compound libraries, and its use for the discovery of novel antiviral drugs directed to RNA targets.

RESULTS

SCAN Principle. This technology is based on the principle that binding of a ligand to a structured RNA causes the stabilization of the structured form. Thus, under conditions of equilibrium between folded and unfolded forms of the target RNA, ligand binding can be measured as a change in their ratio. We have developed a method that uses oligonucleotide hybridization as the means to detect the stabilization of an RNA target upon ligand binding, Figure 1. The hybridization rate of an oligonucleotide to its target sequence is reduced by the presence of stable secondary structures. Therefore, target RNA stabilization by ligand binding will decrease the hybridization rate of an oligonucleotide complementary to the target. Several methods can be used to quantify the extent of hybridization of oligonucleotides, including solution phase methods using fluorescent labels, and affinity capture methods. Figure 1 depicts a SCAN system using streptavidin-based capture for quantitation of hybrids between a radiolabeled RNA target and a biotinylated oligonucleotide.

Proof of principle. We used a 46 nt long ATP-binding RNA-aptamer, AB-RNA (3), Figure 2, as a model system to prove the principle of SCAN. AB-RNA binds ATP with μM affinity. The radiolabeled ATP aptamer was incubated with a biotinylated complementary oligonucleotide and various concentrations of ATP or UTP. After the reaction period, the extent of hybridization was quantified as described in Figure 1. Ligand dependent inhibition of the hybridization reaction was calculated by comparison with a control reaction in the absence of ATP. As shown in figure 2, the rate of hybridization of a biotinylated oligonucleotide to AB-RNA was reduced by 50% at a concentration of 5 μM ATP, whereas UTP had no effect at the same concentration. Similar results were obtained in a solution phase system using a fluorophore labeled RNA target to monitor its hybridization to a cold oligonucleotide by fluorescence quenching. This result demonstrates that the SCAN assay can detect

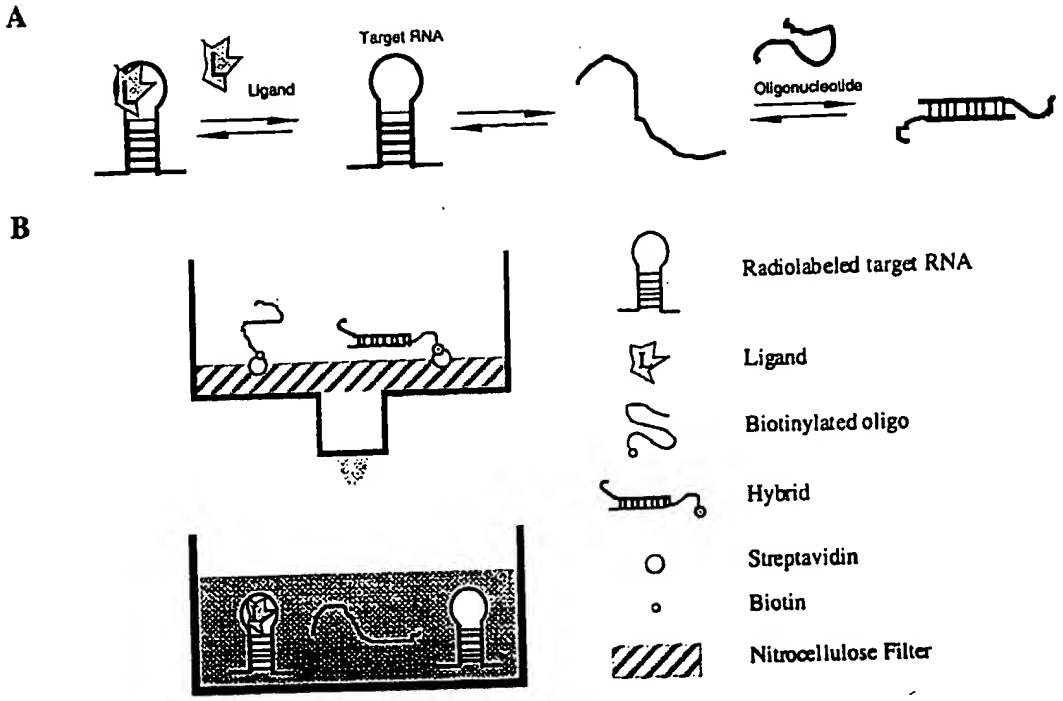

Figure 1. Schematic representation of SCAN. A) Incubation of a structured target RNA with a complementary oligonucleotide results in the formation of an oligonucleotide:target-RNA hybrid. Addition of a ligand stabilizing the folded conformation of target-RNA will disfavor the formation of the hybrid. B) To determine the extent of hybridization of biotinylated oligonucleotides to a radiolabeled target RNA, a streptavidin fusion protein is added and the protein-nucleic acid complexes are quantified after filtration through nitrocellulose. This process is performed in 96-well format using semi-automated stations.

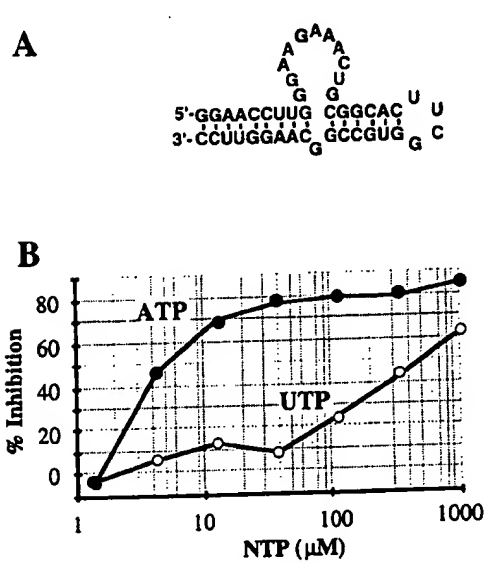

Figure 2. A) ATP-binding RNA aptamer. B) The hybridization extent of radiolabeled AB-RNA to biotinylated oligonucleotide, in the presence of increasing concentrations of ATP, was compared to a control without ATP and the ratio expressed as % of inhibition.

a specific interaction between small molecule ligands and an RNA target. We have successfully developed SCAN assays for several RNA targets including the HIV Tar and RRE elements, the encapsidation signal of HBV, and other regulatory RNA's. An example of SCAN's application to drug discovery is described below.

Application of SCAN to the discovery of anti-HBV compounds. The encapsidation and replication of the HBV genome requires the recognition of a highly conserved RNA structure, epsilon, by the viral polymerase. This RNA structure, Figure 3, located at the 5'-end of the pregenomic RNA has been implicated in multiple functions including pregenomic RNA encapsidation and replication of the viral genome. This process begins with the formation of a stable complex between the viral polymerase and epsilon. This complex is then encapsidated into viral core particles. Replication starts with the protein-primed synthesis of a short oligonucleotide primer using the epsilon bulge sequences as template, Figure 3: positions 10-13, (4-5). The sequence of epsilon is also part of the open reading frame encoding the

pre-core viral protein. The unique structural characteristics, tight sequence conservation, and multiple functionality make epsilon an excellent RNA target for antiviral drugs against HBV.

We have used SCAN technology to screen for specific ligands of epsilon RNA from a diverse compound library with an average molecular weight of 300-400 daltons. All primary hits were confirmed by titration in SCAN reactions in the absence and in the presence of an excess of an unlabeled, non-specific competitor RNA to determine their specificity. Screening of 115,000 compounds yielded 116 compounds with specific affinity for the epsilon RNA. Initial testing of the most active compounds in a cell-based HBV viral replication assay (6) identified 18 compounds with potent antiviral activity. These compounds belong to seven different chemical classes which do not include nucleoside analogs. Subsequent testing of numerous analogs, resulted in the identification of compounds with antiviral potencies similar to that of antiviral nucleoside analogs. Studies to confirm these compound's antiviral mechanism of action are currently underway.

Binding site identification. To further characterize ligand binding to epsilon RNA, at the molecular level, we performed RNAse protection and chemical modification protection experiments. Figure 4 shows the CMCT chemical modification patterns of epsilon RNA in the presence and absence of compound ST, identified by SCAN as an epsilon ligand. The results indicate that the ligand binds to the bulge of epsilon RNA. Binding to the bulge region of epsilon RNA was also observed in DMS modification and nuclease protection experiments, and by thermal melting stabilization.

These observations demonstrate that SCAN can be used for the identification of highly specific ligands of structured RNA targets.

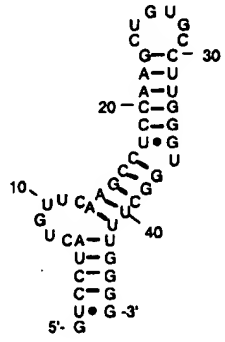

Figure 3. HBV epsil n RNA structure.

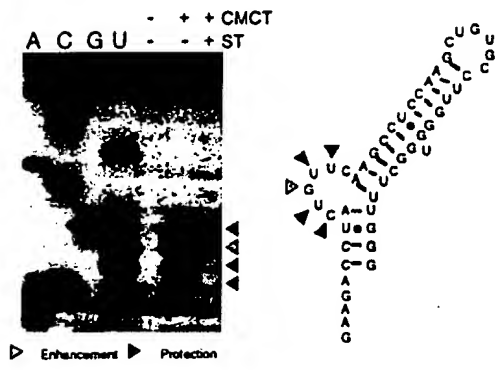

Figure 4. The pattern of modification of uridine and guanine bases with CMCT (1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide) is shown. In presence of ligand ST, (shown to the right), modification protection or enhancement of specific positions is indicated. The modification pattern of the loop and double stranded regions of epsilon RNA were not affected by ST.

DISCUSSION

In this work we have described the use of SCAN to identify specific ligands of HBV epsilon RNA, and demonstrated by independent methods the binding of a ligand to a specific site on the target. We have found that approximately 20% of the epsilon ligands initially selected by SCAN showed antiviral activity in a cell-based HBV replication model. The high frequency of antivirally active compounds among those selected by SCAN suggests that their antiviral activity may occur by the expected mechanism, epsilon binding. A series of experiments to obtain direct evidence of the antiviral mechanism of action are currently under way.

SCAN is an excellent tool that allows the rapid identification of ligands to RNA targets of therapeutic interest. Typical drug discovery efforts involve the development of an *in vitro* functional assay and its use to screen for inhibitors of the activity being measured. In contrast, SCAN does not require the development of an *in vitro* functional assay and ligands that bind any site of the target can be identified, regardless of the target's function(s). Thus, inhibitors for any activities of the target can be identified in a single screen, including inhibitors of previously unknown activities. Using SCAN, a large compound library can be rapidly reduced to a small number of compounds with demonstrated affinity for the target. This small number of compounds can then be directly tested for activity in more cumbersome cell free and tissue culture systems, such as antiviral assays, which are not generally amenable to high throughput screening.

ACKNOWLEDGMENTS

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Exhibit B

Dr. Hanchak and colleagues report that compliance among enrollees jumped from 55.8% during the year before the study to 76.1% after the reminder program. They compared their results with results obtained from a group of HMO enrollees who did not receive the mailings and discovered that vaccination rates in the latter group were only 40.9% in the first year and 52.6% in the second year. Dr. Hanchak also estimates that 144 admissions for acute and chronic respiratory conditions may have been averted by the reminder program.

Vaccination of healthy working adults. In 1995, Dr. Kristen Nichols and colleagues at the VA Medical Center in Minnesota reported that vaccination of healthy working adults by conventional methods in the US would result in a cost-savings of $46.50 per person (1995 *NEJM*: 333:889). Other database and clinical studies also indicate favorable pharmacoeconomic outcomes following vaccination. However, some investigators have argued that a cost-savings may not be realized in all countries where medical programs and insurance, attack rates, cost of vaccines and other factors differ from the US.

***Social biopsy* factor.** Dr. David S Fedson (Pasteur Merieux MSD; Leone, France) and colleagues recently reported on influenza vaccine distribution and national recommendation and reimbursement policies in 22 developed countries for 1995 and 1997. Their research revealed large variations in the levels of influenza vaccine usage. Dr. Fedson referred to a country's influenza vaccination practices as a *social biopsy*; that is, a reflection of its social attitudes as much as a matter of medical expediency. For example, Japan's distribution rate plummeted from a high of over 280 doses per 1,000 residents in the early 1980s to as low as two doses per 1,000 residents in the mid 1990s.

Actual flu vaccine market. The actual vaccine market in the US for the 1997-98 flu season (in terms of actual doses) was estimated to be approximately 70-75 million doses. On average, manufacturers sold influenza vaccine for $18 per ten dose vial, although prices ranged from as low as $15 to as high as $25. The market structure is diffuse, with nearly 50% of vaccine sales made to individual clinics and independent physicians. Major influenza vaccine distributors often relied, in turn, on local wholesale operators to reach the broader market. Sales to large HMOs, hospitals and government agencies represent only 10-15% of the total actual influenza vaccine market. Profit margins were much slimmer due to competition among manufacturers for the high-volume purchasers. Figure 1 presents the estimated US market shares for influenza vaccine manufacturers for the 1997-98 season. All participants were expected to realize a larger share of the US market over last season since Parke-Davis (Morris Plains, NJ) withdrew Fluogen from the market after a voluntary recall of ineffective lots of vaccine during the 1996-97 season. The market-leading Connaught Laboratories (Swiftwater, PA) is expected to gain the most market share this season.

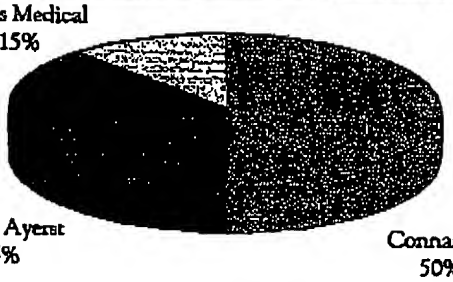

Figure 1. Estimated US Market Shares for Influenza Vaccine Manufacturers — 1997-1998 Season.
Source: D&MD.

TECHNOLOGY & STRATEGY

The Current Status of High-Throughput Screening and Outlook for the Future, Part I

- This is Part 1 of a two-part article which assesses the emerging high-throughput screening (HTS) marketplace as it applies to drug discovery in the large-cap pharmaceutical companies.
- The following topics are reviewed in Part 1: (1) historical overview and definitions employed in the HTS business, (2) segmentation of the markets as they evolve, (3) technology platforms, (4) competitive environment, and (5) an analyst's view of the industry with respect to market opportunity, timelines and emerging trends.
- In Part 2 of this article, which will appear in the May, 1998 issue of Drug & Market Development, a case study of a high-throughput screening company located in the San Francisco Bay area will be presented. This article will highlight the business models and target markets explored by the company, serving to illustrate the challenges and opportunities facing the emerging HTS industry.
- This article was prepared by strategic analyst Enal S Razvi, Ph.D. (erazvi@vax.clarku.edu) of Portola Valley, CA.

Historical Overview

Screening of small molecules in the attempt to discover pharmaceutical targets is not a new endeavor. The large-cap pharmaceutical companies have been screening historical file compounds in their databases for years in the attempt to discover new drugs, either for new disease targets or in the effort to improve existing therapies. With the explosion in genomics and the realization that pharmaceuticals in the future will be built around the patient's genetic constitution, big pharma has embraced HTS as a *bona fide* discipline. HTS and ultra high-throughput screening (UHTS) are now becoming essential components of R&D in the big pharmaceutical companies. These companies are the end-users of the HTS/UHTS technologies. They have both the need for sample and data processivity as well as the financial resources to underwrite the R&D efforts being performed at the smaller biotechnology outfits. Table 9 summarizes the historical record of the screening enterprise, as applied to compound screening in pharmaceutical discovery.

Scope and Definition

Even though screening has been performed in the large pharmaceutical companies for decades, only in the last few years has its role been emphasized to the degree that companies are now built around screening products or services as their s le offering. This article focuses on screening at the high-throughput and ultra high-throughput levels as performed by the end-users. HTS and UHTS are operational definitions reflecting the total throughput (or processivity) of the screening technology employed. The current definition (year-end 1997) is as follows:

- HTS: 10,000-100,000 samples processed per day
- UHTS: >100,000 samples processed per day

Segmentation of Evolving Markets

There is no doubt that screening for drug discovery is a rapidly growing discipline. Screening indeed works and is the origin of most drug candidates currently in the pipeline, as well as those already on the market. Furthermore, screening is a cost-effective way to drug-discovery. Given the current climate of the biopharmaceutical industry, with immense excitement generated by genomics and bioinformatics as means to discovering novel genetic targets associated with specific diseases, HTS/UHTS is at the forefront of drug discovery efforts.

At the present time, the market for screening in drug discovery is dominated by the use of the microwell-based platform. This technology, together with competing technologies, is discussed in more detail later. A more recently developed and emerging technology employs a chip-based approach by using microchannels on what have been termed *biochips* for carrying out chemical reactions in a high-throughput manner (see D&MD Report #1901 entitled *Biochip Technologies and Applications* for a comprehensive review of the technology and current market). Biochips promise to be the technology platform for the future of drug discovery. See Table 10 for a breakdown of the HTS/UHTS marketplace.

Raison d'être for HTS

So why all the excitement in the discipline of high-throughput screening of compounds, either in the development of biologics (i.e. bioactive, naturally-occurring compounds) or small-molecule agonists or antagonists of particular intracellular targets? The following list presents some of the reasons behind the increased interest in HTS in recent years:

- The genomics enterprise—whose *raison d'être* is to provide genetic elements implicated in various physiologic or pathologic phenotypes—promises to reveal hundreds, and perhaps even thousands, of genetic elements associated with particular diseases. The entire genomics enterprise, namely structural and functional genomics, aims to discover and provide biochemical functionality to hitherto unknown genetic elements implicated in disease. All of these elements ultimately need to be tested against candidate small molecules, and this fuels the need for HTS.
- Combinatorial chemistry, the discipline of constructing related molecules on a single chemical backbone, provides entire libraries of compounds from which lead compounds must be identified. Current estimates of the size of combinatorial libraries are in the hundreds of thousands of compounds per library. This number is expected to grow to several million in the next two to four years. As combinatorial libraries grow, so does the need for screening them.
- Many of the therapies that involved *easy* target molecules have already been exploited, giving rise to the beta blockers and such which are currently used in the clinical setting. For more complex diseases involving the activity of multiple gene products, the information content per screen needs to increase. In other words, the end-user needs to be able to evaluate the expression of multiple targets in a single screen, or take multiple measurements off a single biochemical reaction. Therefore, in addition to increases in sample throughput, the screens of tomorrow will also need to be informationally dense. Again, the emphasis is placed upon the data content *of the screen*.

Table 11 presents the evolution of drug-discovery technology in order to illustrate the increased need for screening in contemporary times. Note the use of genomics and combinatorial chemistry as essential tools for contemporary drug-discovery efforts, and the attendant need for HTS.

Table 9
Historical Record of Compound Screening in the Biopharmaceutical Industry

1985
• 10,000 samples per year • Assays performed in test-tubes • Very little automation • Screening was the rate-limiting step in drug-discovery
1990
• 100,000 samples per year • Assays performed in microtiter (96-well) plates • Workstation-based automation • Compounds to screen served as the rate-limiting step
1995
• 500,000 samples processed per year • Assays performed in microtiter (96-well) plates • Workstation-based automation • Targets (proteins) to screen served as the rate-limiting step
2000
• Several millions of samples processed (screened) per year • High-density, smaller volume, high information content assays • Assembly line-based automation • Rate-limiting step is the characterization and analysis of leads generated.

Source: Lasure & Crawford, Inc.

McKinsey & Co. recently published an analysis of the breakdown of total costs of drug discovery and the impact of technological advances in terms of time and cost-savings. Table 12 presents the results of this analysis in quantitative terms. It is apparent from Table 12 that 45% of total costs of drug discovery and development are attributable, in some part, to the screening enterprise. Furthermore, given the inefficiencies in time and reagent consumption in the screening process, technological advances in the screening process have the potential of trimming 70-90% off of the time and costs associated with the screening effort (valued at approximately 45% of total drug development costs). The following section presents a summary of the HTS technologies currently available as well as emerging trends.

Technology Platforms for HTS/UHTS

Ultimately, it is big pharma that is underwriting the R&D enterprise of HTS/UHTS, thus making them the primary end-users of this technology. There are two elements big pharma is looking for in the development of HTS/UHTS systems: (1) sample processivity, and (2) information content per sample processed. Sample processivity may be defined as the number of discrete screenings capable of being performed per machine working day. Table 13 examines the sample throughputs of various screening technologies.

HTS and UHTS technologies seek to overcome the bottleneck in drug discovery by increasing assay throughput (sample processivity) per unit time. So how to increase assay throughput? The following section presents the current solution to this problem.

Microwell-based. One of the ways to increase sample processivity is to employ the use of a higher number of wells per assay plate (of a defined footprint). Therefore, each well has a smaller volume and the same robotics machinery can screen a larger number of discrete samples in a given time frame. The current standard of HTS is to use the 96-well plate. However, several companies are now employing plates with a higher well density—384-wells per plate. The emerging technology in microwell based screening is to use plates with 1,536-wells. However, these are still experimental and are not used currently in the HTS/UHTS enterprise. On the horizon in microwell-based screening is the 3,456-well plate from Aurora Biosciences (La Jolla, CA)—the Nanoplate™. This plate format will enable UHTS with sample processivities of more than 100,000 samples per day. Note that in the microwell-based screening format, each well generates one data point per unit time. All those involved in pharmaceutical drug discovery at the present time are utilizing micro wells of some density (density defined in terms of wells per plate). Molecular Devices Corp. (Sunnyvale, CA) produces the Fluorometric Imaging Plate Reader (FLIPR), which is currently the only high-throughput live cell-screening system on the market capable of processing 10,000 samples per day.

The bulk of pharmaceutical screening is primary screening—determining whether an agonist or antagonist affects a given target. This is a binary screen and amenable to the micro well-based format described above. However, once candidate compound(s) have been identified from a primary screen, the subsequent characterization of these lead compounds is an information-intensive exercise. Often, a large number of discrete pieces of data need to be accumulated to arrive at the conclusion of whether or not a given *hit* is worth pursuing further in animal and preclinical studies. So, what technologies on the horizon can give rise to information-dense HTS? The following section sheds some light on this issue.

Microchannel-based. Until now we have considered screening a process which is not dynamic—each sample processed yielding only one data point. Given the need for increased throughput of data and information, the opportunity exists in the marketplace for technologies which enable greater amounts of data acquisition per unit time. Microchannels etched into a solid substrate provide the possibility of movement of fluids via electric fields. Two factors contribute to this: (1) electrophoretic movement of charged molecules in the fluid (such as nucleic acids with a net negative charge), and (2) electrokinetic movement of the fluid itself in the channel. Thus, by the application of appropriate electric fields to the ends of microscopic channels, fluids may be moved around.

The biochip is a solid substratum, such as glass or plastic, with microchannels etched into it. Potential differences may be applied across the channels to move fluids within the channels as well as achieve dynamic mixing of fluids within the channels. Biochips provide a very powerful combination of wet biochemistry with computer control (the mixing of the reagents in the channels is mediated by voltage differences applied in sequence under microprocessor control). So, what can biochips actually do for the biopharmaceutical community? At this stage, biochips are still in the R&D phase, but the first prototypes are expected to reach the end-users (big pharmaceutical and diagnostics companies) around 2Q'98, at the earliest. What will drive the HTS/UHTS market to adopt microchannel-based devices for screening candidate molecules? The following list presents a set of arguments for the penetration of microchannel-based technology into the pharma HTS/UHTS marketplace:

- Assay volumes are in the pL range, thereby decreasing cost of consumables for HTS and extending the useful life of combinatorial libraries. It is estimated that the average cost of screening using microwells is currently around $1 per screen. Biochips may be able to bring the cost down to $0.10 *per data point.*

Table 10
Segmentation of the HTS/UHTS Marketplace

Segment 1 Microwell-Based HTS/UHTS
• HTS is currently practiced; UHTS is expected to hit the marketplace by 1999-2000 • Current technology platform • Performed in microwells of density 96-wells per plate; densities of 384-wells per plate are now hitting the market and should begin to penetrate the market by 3Q'98 • UHTS will be performed in 2 microliter (μL) wells (with 1 μL of sample per well) at a density of 3,456-wells per plate
Segment 2 **Microchannel-Based HTS**
• Currently in the R&D phase; nothing on the market as yet • Prototypes should be shipped to *preferred customers* by 2Q'98 • Sample volumes are in the picoliter (pL) range • Data throughput is expected to be 10-15 times greater than conventional microwell-based assays

Source: D&MD.

Table 11
Drug Discovery Technologies—Then and Now

1920s
• Major drug classes were the antibiotics • Sources were medicinal chemistry, natural products • Target identification was via human physiology • Targets of therapies were micro-organisms
1950s
• Major drug classes were cardiovascular drugs, anti-hypertensives, diuretics, anti-arrhythmics, psychotropics • Sources were again from natural products • Target identification was via biochemistry and cell biology • Targets were enzymes and receptors
1980s
• Drug classes were composed of recombinant proteins, monoclonal antibodies • Sources were tissue culture and genetic engineering (recombinant DNA technology) • Target identification was via molecular biology • Targets were genes and proteins
1990s—2000 and beyond
• Drug classes are composed of peptides and small molecules • Sources are combinatorial peptide synthesis and combinatorial chemistry • Target identification is via structural genomics, functional genomics, HTS and the use of genetically-constructed animal models • Targets are genes, genetic regulatory elements and intracellular signal transduction pathways

Source: Volpe Brown Whelan & Co.

Table 12
Costs Involved in Drug Discovery/Development and Impact of Technological Advances on Savings

Understanding the Disease
• Cost associated = 5% of total • Impact of advances in terms of time savings = 50% • Impact of advances in terms of cost savings = 0%
Selection of Target
• Cost associated = 5% of total • Impact of advances in terms of time savings = 70% • Impact of advances in terms of cost savings = 70%
Designing the Primary Screen
• Cost associated = 10% of total • Impact of advances in terms of time savings = 10% • Impact of advances in terms of cost savings = 10%
Screening and Identification of Hits
• Costs associated = 15% of total • Impact of advances in terms of time savings = 90% • Impact of advances in terms of cost savings = 90%
Converting Hits to Lead Compounds
• Costs associated = 20% of total • Impact of advances in terms of time savings = 70% • Impact of advances in terms of cost savings = 70%
Converting Lead Compounds to Development Candidates
• Costs associated = 45% of total • Impact of advances in terms of time savings = 10% • Impact of advances in terms of cost savings = 10%

Source: McKinsey & Co.

- Once the reagents are loaded onto the chip, a variety of configurations for mixing and measuring can be achieved using extrinsic [to the chip] computer control—increasing the flexibility of the assays that may be performed.
- The information content the microchannel-based system can deliver per given sample is much higher than that which may be achieved with microwells. In this way, a typical run using an enzyme, its substrate and a set of inhibitory compounds can determine the Km of the enzyme, and in the same run determine the Ki of the various inhibitors tested, thus increasing the informational density of the screen. Ultimately, the unit shipment (in terms of pricing) from the HTS enterprise will be units of information, rather than the plastic chips themselves.
- The footprint (size and shape) of the microchannel-based devices is comparable to microwell plates. In this way, robotics and other machinery that many companies already have in place will be easily adaptable to the next format. This will reduce the cost factor involved in the adoption of this new technology and will thus create a conversion market—from the microwell-based format to the microchannel-based format.

In summary, therefore, there are two platforms for HTS/UHTS—microwells and microchannels. Currently, 100 percent of the screening market is dominated by microwell-based screening. Microchannel-based devices are still in the developmental stage and are expected to make a debut in the next 8-12 months. In the final section of this article, an analyst's view of the HTS/UHTS market is presented with predictions for the future of this enterprise.

The Competitive Landscape

This section presents a summary of three major players in the microwell-based HTS/UHTS marketplace, as well as two major players in the microchannel-based (biochip) HTS marketplace.

Aurora Biosciences, Inc. is a dedicated HTS company which completed an initial public offering (IPO) of 4,000,000 shares (priced at $10 each) in the summer of 1997, raising $40 million on Wall Street (NASDAQ: ABSC). Aurora is pursuing the business model whereby the company develops its proprietary UHTS system in-house and partners with select big pharma firms to finance their in-house R&D operation. Once the UHTS system (with expected sample processivities of >100,000 samples per day) is completed, it will be shipped to Aurora's partners. Aurora's technology hinges on the use of extremely high-density microwells (their Nanoplate will have 3,456-wells) and cell-based screening assays. Shipments of the completed UHTS from Aurora are expected in 4Q'99 or 1Q2000. Ultimately, Aurora is aiming to target the cell-based HTS/UHTS marketplace—the upper echelon of screening.

Molecular Devices Corporation is a well-known player in the laboratory automation marketplace with spectrophotometer and microwell plate-readers (NASDAQ: MDCC). Molecular Devices is participating in the emerging HTS market for drug discovery by shipping its product, FLIPR, to the pharmaceutical marketplace. FLIPR is the only true high-throughput live cell-screening product on the market today capable of delivering sample processivities of approximately 10,000 samples per day.

LJL Biosystems (Sunnyvale, CA) is entering the field of HTS. LJL was founded to produce instrumentation for clinical diagnostics laboratories, but has changed its focus to take advantage of the growing interest in HTS in the pharmaceutical community. In 3Q'97, LJL introduced its 96-well and 384-well systems using fluorescence readouts for HTS (Analyst™ system). Earlier in 1997, LJL completed a private placement round of financing raising $9 million through Montgomery Securities. LJL is currently privately-held.

Caliper Technologies Corporation (Palo Alto, CA) is developing its prototype LabChip™, a version of the biochip capable of performing biochemical reactions in solid substrata. The company has forged an alliance with Roche (Nutley, NJ) for the development of an UHTS based on microchannel technology. Furthermore, Caliper plans to perform HTS in-house using its channel-based platform. Thus, it appears that Caliper is pursuing two business models: (1) shipping chips and the associated hardware to the research community (either academic or industry), or (2) offering a fee-for-service business. Caliper is constructing clean rooms with GMP-certification for the in-house manufacture of chips, and the company plans an IPO in the next 8-12 months.

Soane Biosciences (Hayward, CA) is developing HTS systems for drug discovery based on microchannel technology. The company is pioneering the use of disposable plastic chips containing microchannels in various formats for performing biochemical reactions *in situ*. Soane has formed a collaboration with Johnson & Johnson (New Brunswick, NJ) for developing chips for HTS in drug discovery, and another with Hitachi (Tokyo, Japan) for the exploration of plastic biochips in molecular diagnostics. Soane's business model is to champion its strengths in

Table 13
Sample Throughput Capacities of Various Screening Technologies

Technology	Throughput (assays/day)
Manual	1,000
Semi-automated	5,000
HTS (low-end)	10,000
HTS (high-end)	100,000
UHTS	>100,000

Source: D&MD.

performing biochemical reactions as well as DNA separations in plastic for targeting the HTS and diagnostics markets.

Market Opportunity, Timeline and Emerging Trends

HTS is currently a very hot topic in the pharmaceutical community. Major players are companies with excess cash and a tight bureaucratic structure. Therefore, it is easier for them to buy or license technology than it is to develop it for themselves. For big pharma, HTS is at the core of the entire drug discovery enterprise, and companies are willing to bet on the success of dedicated screening outfits such as Aurora Biosciences to deliver the processivity and throughput that they require. Jürgen Drews (former President of Global Research; Hoffmann La-Roche) has calculated that the drug discovery enterprise as it stands today at big pharma is incapable of sustaining the 15% or so annual growth in revenues the capital markets require for these firms to sustain their valuations. So, what is the solution?—A move toward outsourcing drug discovery projects to dedicated biotechnology outfits.

HTS is a growing enterprise and big pharma's need to screen is limited only by the available technology. If a system with higher levels of throughput were to be presented today, big pharma would pay for it. One estimate for the total market value of HTS ranges in the billions of dollars. After all, if estimates that pharma spent about $18 billion on R&D in 1997 are correct, and 15-17% of that amount is dedicated to screening, over $3 billion was spent in 1997 on screening alone—HTS being one of the components of the total screening enterprise. So where is the field heading? With a huge market opportunity, the expectation is for more players to enter the market. Some will be small outfits dedicated to performing specific services (in a fee-for-service model), which may eventually merge or be acquired by big pharma. Industry sources report that HTS on targets revealed by the genomics enterprise has not begun as yet in the big pharma community. It is expected, however, that this will commence in the next 8-12 months. With the introduction of UHTS and the use of genomics-derived targets, the HTS industry is expected to take an upward inflection around 3Q'99-1Q2000 and continue upward with the use of biochip-based screening and the resultant exponential increases in data acquisition.

Progress Towards Clinically Successful Xenotransplantation

- As a result of the large, unmet need for transplant tissue, many biotechnology companies have sprung up to address the challenge of finding alternative sources of tissue, and the field of xenotransplantation has expanded rapidly over the past five or so years.
- Some approaches that may contribute to the future viability of xenotransplantation as a clinical practice include the inhibition of complement binding, with various approaches being taken by T Cell Sciences, Alexion Pharmaceuticals and Nextran, and the elimination of *offending* antigens which contribute to xenograft rejection, an approach also being explored by Alexion and Nextran, as well as BioTransplant, Inc.
- This article reviews some of the latest advances in the field of xenotransplantation, as well as some of the most pertinent safety issues that have recently arisen in Europe and the US.
- This article was prepared by Chester A. Bisbee, Ph.D., Andover, MA.

Introduction

The era of organ transplantation began in 1954 when Dr. Joseph Murray and his surgical team successfully transplanted a kidney from one identical twin into another. In the intervening 44 years, organ transplantation has become a standard medical procedure. In fact, some less dramatic forms of tissue transplantation, such as blood transfusion and bone or ligament replacement, are commonplace clinical procedures.

However, organ transplantation remains expensive. Renal transplants cost around $50,000 per operation. Yet, transplantation remains a cost-effective treatment for end-stage renal disease because patients undergoing hemodialysis, the alternative treatment, expend this amount for each year of dialysis. In contrast, after the initial transplant, annual costs for treatment of transplant patients usually do not exceed $10,000, which includes the necessary payments for immunosuppressants. In addition, as important as the direct cost savings, the social and psychological advantages of transplantation are great.

Although between 18,000 and 19,000 kidney transplants will be performed this year in the US (see Table 14), large organ transplantation continues to be plagued by a shortage of donors. Well over twice as many patients are registered in organ banks as will receive transplants this year. An even greater number of potential patients who could also benefit from organ transplants, but who are not prime candidates for this procedure because of other medical conditions, are not even registered. Additionally, as surgical and related biomedical technology continues to advance, the number of organs needed will increase at a rate that greatly outpaces that of organ supply. Estimates of the number of individuals who could benefit from a kidney transplant in the US, Europe and Japan are shown in Table 15. Due to the drastic discrepancies between those who need organs and the number of donor organs available, two major issues are continuously being addressed by clinicians: (1) rationing of these currently scarce resources and (2) increasing donor organ availability.

Donor organ availability can be increased in two ways—either more organs can be donated or alternative sources can be developed. Increased numbers of donated organs can be achieved either through legislation encouraging voluntary donation or creation of a market for organs and tissues. Most European countries that have adopted legislation to encourage donation have seen only marginal increases in organ availability. Similarly, the creation of an open market would most likely increase distrust and cause great concerns relating to fairness,

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